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## Influence of sampling strategies on the monitoring of cyanobacteria in shallow lakes: Lessons from a case study in France

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1 **Influence of sampling strategies on the monitoring of cyanobacteria in**  
2 **shallow lakes: lessons from a case study in France**

3

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9

9 **Abstract**

10 Sampling cyanobacteria in freshwater ecosystems is a crucial aspect of monitoring programs  
11 in both basic and applied research. Despite this, few papers have dealt with this aspect, and a  
12 high proportion of cyanobacteria monitoring programs are still based on monthly or twice-  
13 monthly water sampling, usually performed at a single location. In this study, we conducted  
14 high frequency spatial and temporal water sampling in a small eutrophic shallow lake that  
15 experiences cyanobacterial blooms every year. We demonstrate that the spatial and temporal  
16 aspects of the sampling strategy had a considerable impact on the findings of cyanobacteria  
17 monitoring in this lake. In particular, two peaks of *Aphanizomenon flos-aquae* cell  
18 abundances were usually not picked up by the various temporal sampling strategies tested. In  
19 contrast, sampling once a month was sufficient to provide a good overall estimation of the  
20 population dynamics of *Microcystis aeruginosa*. The spatial frequency of sampling was also  
21 important, and the choice in the location of the sampling points around the lake was very  
22 important if only two or three sampling points were used. When four or five sampling points  
23 were used, this reduced the impact of the choice of the location of the sampling points, and  
24 allowed to obtain fairly similar results than when six sampling points were used. These  
25 findings demonstrate the importance of the sampling strategy in cyanobacteria monitoring,  
26 and the fact that it is impossible to propose a single universal sampling strategy that is  
27 appropriate for all freshwater ecosystems and also for all cyanobacteria.

28

29 Keywords: sampling strategy, cyanobacteria, spatiotemporal dynamic, *Microcystis*  
30 *aeruginosa*, *Aphanizomenon flos-aquae*

31

## 31 **1 Introduction**

32 Due to eutrophication and, to a lesser extent, to climatic changes (Markensten et al., 2010;  
33 Paerl and Huisman, 2009) cyanobacterial blooms seem to be increasing in freshwater  
34 ecosystems worldwide. These blooms severely disrupt the functioning of these ecosystems  
35 and potential water use. Furthermore, many cyanobacterial species are able to produce a  
36 variety of toxic metabolites, which can be harmful to both human (Kuiper-Goodman et al.,  
37 1999) and animal (Codd et al., 2005) health. For these reasons, numerous attempts have been  
38 made in the last 20 years to elucidate the factors that control cyanobacterial blooms and toxin  
39 production, and thus to make it possible to evaluate better the health risks associated with  
40 bloom events. From all these studies, it is clear that the spatial distribution of cyanobacteria in  
41 freshwater ecosystems can display marked horizontal and vertical variations (Porat et al.,  
42 2001; Welker et al., 2003). Moreover, by means of a real time PCR analysis of a gene  
43 involved in the biosynthesis of microcystins we have shown that considerable fluctuations can  
44 also occur in the proportions of potentially microcystin-producing and non-producing cells  
45 during the course of *Microcystis aeruginosa* blooms (Briand et al., 2009). Similar results have  
46 been found for various different *M. aeruginosa* populations located in the same geographic  
47 area (Sabart et al., 2009), which makes it difficult to manage the health risks associated with  
48 these events.

49 All these studies indicate that the sampling strategy used for monitoring cyanobacteria  
50 is a critical aspect, both in basic research on cyanobacteria, (e.g. investigation of the factors  
51 and processes involved in the development of the blooms), and in applied research, (e.g.  
52 implementing monitoring programs of these microorganisms in freshwater ecosystems used to  
53 provide drinking water or for recreational activities). In recent years, new tools have been  
54 tested with the intention of improving cyanobacterial sampling, for example, remote sensing

55 reconnaissance to determine the horizontal distribution of cyanobacteria in freshwater  
56 ecosystems (Hunter et al., 2009), or spectrofluorometric probes to reveal the vertical  
57 distribution of these cyanobacteria in the water column (Leboulanger et al., 2002). Moreover,  
58 these spectrofluorometric probes and other sensors have now been integrated into buoys, to  
59 provide real-time monitoring of cyanobacteria in freshwater ecosystems (Le Vu et al., in  
60 press).

61         However, despite the great potential interest of these tools, their cost will remain  
62 prohibitive for their routine use in the foreseeable future, and most of the monitoring  
63 programs worldwide for the survey of cyanobacteria will continue to be based on more  
64 conventional methods for some years to come. Taking discrete samples of various volumes of  
65 water taken from the shoreline of ecosystems is probably the method one most often used in  
66 studies. Unfortunately, as a result of spatial and temporal differences in the distribution of  
67 cyanobacteria, this approach can often provide a very poor estimation of cyanobacterial  
68 abundance and, consequently, of the associated health risk. We therefore need to devise  
69 simple sampling strategies for the low cost monitoring of cyanobacteria in shallow lakes. In  
70 an attempt to do this, we performed intense spatiotemporal monitoring of cyanobacteria in a  
71 shallow lake known to experience cyanobacterial blooms every year.

72

## 73 **2 Materials & Methods**

### 74 **2.1 Study site**

75 This study was performed in a shallow lake named Place (0.08 km<sup>2</sup>, 2.5 m max depth,  
76 45°43'N, 4°14'E) located in the plain of Forez (Central France), (Fig. 1). This lake is used for  
77 extensive fish production and its trophic status is eutrophic to hypereutrophic (OCDE, 1982).  
78 *Microcystis aeruginosa* blooms occur every summer.

79

## 80 **2.2 Data acquisition**

### 81 *2.2.1 Sampling strategy and cell counting*

82 In order to assess the variations in the horizontal distribution of cyanobacteria in this pond, we  
83 monitored six sampling points located around the lake at one meter from the shore (V1-V6;  
84 Fig. 1). The water depth in each of these sampling points was around 1 meter. Samples were  
85 taken every two days, between 09:00 and 10:00 a.m., from early June 2008 to early October  
86 2008. The first 40-centimeters of the water column were sampled using a watersampler  
87 (Uwitech, Austria). This water sample was shaken and then divided into two 1-L bottles, one  
88 liter being stored at room temperature with Lugol's iodine solution, and the other at 4°C.

89 In order to evaluate the vertical distribution of cyanobacteria in the water column, we  
90 performed a 22-hour survey (from 4:30 p.m. August 4, 2009 to 2:30 p.m. August 5, 2009), of  
91 the variations of cyanobacterial biomass at five sampling points (A-E; Fig. 1) using a BBE  
92 Algaetorch (Moldaenke, Germany). This torch is based on the same principle as the BBE  
93 spectrofluorometric probe (Beutler et al., 2002), but provides only an estimation of the  
94 concentrations of cyanobacteria and total chlorophyll in water. Every hour, the torch was  
95 immersed to a depth of 20 centimeters at the five sampling points, and triplicate  
96 measurements were performed in each point.

97 The cyanobacterial cell concentrations were estimated using a Nageotte cell and an  
98 optical microscope, as described in Brient et al. (2008). For each rectangular area, we counted  
99 at least 400 cells of each cyanobacterial species.

100

### 101 *2.2.2 Meteorological data*

102 The speed and direction of wind during our study were obtained from the Météo France  
103 meteorological station at St Etienne-Bouthéon (4°18'E – 45°32'N). The wind direction rose

104 for this station is given in Supplemental Figure 1, and shows that the two dominant wind  
105 directions were NW and SE. The direction of winds blowing from 240° – 60° was classified  
106 as NW, and that of winds blowing from 60° – 240° as SE.

107

### 108 **2.3 Data analysis**

109 The spatial distribution of cyanobacteria in the lake was represented using Surfer (v. 7.0,  
110 Golden Software Inc.), and statistical analyses (Wilcoxon test, Spearman correlation) were  
111 performed using the R package version 2.10 (R development core Team, 2010).

112

## 113 **3 Results**

### 114 **3.1 Change over time in the population dynamics of the two dominant cyanobacterial** 115 **species**

116 Two cyanobacterial species, *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*,  
117 dominated the phytoplankton community during the summer of 2008. The population  
118 dynamics of these two species displayed very contrasting patterns (Fig. 2). The population  
119 dynamics of *Microcystis aeruginosa* was characterized by a steady increase in the cell  
120 abundance from June to August, apart from a brief dip in the middle of July. The maximum  
121 population was reached on August 21 (264,000 cells/mL), and subsequently the cell  
122 concentration remained stable until the end of September, and then decreased in October. In  
123 contrast, the population dynamics of *Aphanizomenon flos-aquae* were much more chaotic,  
124 with the cell abundance reaching two very high and short-lived peaks in July  
125 (400,000 cells/mL on July 17, and 560,000 cells/mL on July 23).

126

### 127 **3.2 Influence of sampling frequency on the estimation of the population dynamics**

128 Our assessment of the changing population dynamics of the two cyanobacteria were obtained  
129 using a very frequent high temporal sampling regime (every two days), which would not be  
130 practicable in the context of normal monitoring programs. In order to evaluate the impact of  
131 the sampling frequency, we simulated weekly, twice-monthly and monthly sampling  
132 frequencies to our data set. The results of these simulations are shown in Fig. 3 and 4. From  
133 this figure, we can see that changes in *M. aeruginosa* cell abundance over time would have  
134 been fairly accurately estimated at all these sampling frequencies. Moreover, for all sampling  
135 frequencies, the quality of the estimation of the *M. aeruginosa* population dynamics was not  
136 influenced by choice of the first sampling date (Fig. 3). In contrast, the population dynamics  
137 of *A. flos-aquae* would have been badly or even very badly estimated by using weekly, twice-  
138 monthly and monthly sampling frequencies (Fig. 4). We would only have detected both  
139 *A. flos-aquae* peaks in one of the three trials testing the weekly sampling strategy, and we  
140 would never have detected these peaks with twice-monthly and monthly sampling  
141 frequencies.

142

### 143 **3.3 Evolution of the horizontal distribution of cyanobacteria in the lake during the** 144 **bloom**

145 As shown in the video (Supplemental Fig. 2), the horizontal distribution of both cyanobacteria  
146 displayed marked variations during the course of the study. Moreover, when the spatial  
147 distributions of the two species at the same sampling dates were compared, it could be seen  
148 that similar or contrasting patterns in the horizontal distribution of *M. aeruginosa* and *A. flos-*  
149 *aquae* cells would have been found, depending on the dates chosen (some examples are  
150 provided in Fig. 5).

151 In order to obtain a better picture of this spatial variability in the cell concentrations of  
152 the two species, we estimated the coefficients of variation in the mean abundance for each



153 sampling date and for each species from the results obtained at the six sampling points  
154 (Fig. 6). These coefficients were usually higher for *A. flos-aquae* than for *M. aeruginosa*  
155 (Wilcoxon test,  $p=3.25 \cdot 10^{-05}$ ), suggesting that the horizontal distribution of *A. flos-aquae* was  
156 more variable. Finally, there was no correlation (Spearman coefficient) between the  
157 coefficient of variation and the mean cell abundance for *Aphanizomenon*, and only a weak  
158 correlation was found for *Microcystis* (Spearman coefficient,  $p=0.003$   $r=-0.4$ ; Supplemental  
159 Fig. 3).

160 In order to find out whether wind speed/direction could account for the variations in  
161 the horizontal distribution of cyanobacterial cell abundance in the lake, we recorded in a first  
162 time, for each species and for each sampling date, the sampling point (out of the six) at which  
163 the highest cell abundance was detected. We then constructed a table in which we related  
164 these findings to the wind direction and speed in the five hours before the sampling, knowing  
165 that only data with wind speed values  $\geq 2.0$  m/s were taken into consideration. For *M.*  
166 *aeruginosa*, the detection of the highest cell abundances in the southernmost sampling points  
167 V2 and V3 were associated with winds blowing from the NW (Table 1), whereas those at the  
168 V1 and V4 sampling points were more surprisingly associated with winds from the SE. High  
169 cell abundances in the northern most sampling points V5 and V6 were equally associated with  
170 winds from NW and SE. For *A. flos aquae*, the results were more complicated, and no  
171 obvious link could be seen between the direction of the wind and the distribution of the  
172 cyanobacteria (Table 1). The same analyses were performed by taking into account the wind  
173 data one and two days before sampling (instead 5-10 hours before sampling), but no obvious  
174 relationship was detected (data not shown).

175

### 176 **3.4 Influence of the number of sampling points on the estimated cyanobacterial cell** 177 **abundances in the lake**

178 The cyanobacterial cell abundances in the shallow lake were estimated by calculating the  
179 average value for the six sampling points (see Fig. 1). In order to determine the number of  
180 sampling points required to obtain a good estimation of cyanobacterial cell abundances in the  
181 lake, we compared the estimations of cell abundance based on using samples from just one,  
182 two, three, four or five sampling points with that based on all six. To do this, we calculated  
183 the correlation coefficients (Spearman) between the estimations based on the six sampling  
184 points and those based on one to five sampling points for each species (Fig. 7). We considered  
185 all possible combinations of points, and the results are classified in the figure on the basis of  
186 increasing order of r values within each combination of groups. For both species, we found  
187 that the estimations of cell abundances based on only one or two sampling points were  
188 generally rather badly correlated with those obtained using all six sampling points. On the  
189 other hand, it appeared that good correlations (around or  $> 0.9$ ) were obtained when at least  
190 three sampling points were used, but also that the variations due to the choice of the sampling  
191 points was still considerable when only three sampling points were used.

192 In order to find out which combinations of sampling points provided the best results  
193 when only two or three sampling points were used, we classified all the possible combinations  
194 of points. To do this, we added the rank of each combination of sampling points obtained for  
195 the two species (*M. aeruginosa* and *A. flos-aquae*). From Figure 8, we can see that the best  
196 estimations obtained using only two or three sampling points were provided by combinations  
197 in which the sampling points used were on the shore opposite to the prevailing wind direction  
198 over the lake.

199

### 200 **3.5 Diel variations in the subsurface cyanobacterial biomass in the lake**

201 Finally, we carried out a 24-hour estimation of the variations in the total cyanobacterial  
202 biomass in the subsurface water (20 cm depth) of the lake, at five sampling points using the

203 BBE torch (A-E, see Fig. 1). As shown in Fig. 9, there was a steady fall in the cyanobacterial  
204 biomass at all sampling points during the afternoon and evening, and conversely an increase  
205 late at night and in the morning. Moreover, the differences in biomass between the five  
206 sampling points were smaller during the night than during the day, as was the standard error  
207 (three measurements per sampling point). A multidimensional scaling analysis performed on  
208 the same values confirmed these observations, with all the night sampling times being  
209 grouped together, whereas the sampling times during the day were much more scattered (Data  
210 not shown).

211

## 212 **4 Discussion**

213 As far as we are aware, this is the first attempt to investigate the influence of sampling  
214 strategies on the evaluation of spatial and temporal variations in cyanobacterial abundances in  
215 shallow lakes, which constitute unstable and complex ecosystems. These lakes are used by  
216 humans for numerous activities, including recreational activities and the supply of drinking  
217 water, which makes the monitoring of cyanobacteria in such ecosystems of particular  
218 importance, especially as part of the evaluation of the health risks linked to cyanobacterial  
219 blooms and their toxins. Sampling strategy is also very important in the context of basic  
220 studies, because the quality of sampling has a major impact on the quality of the final results.

221 In this study, we found that the sampling frequency required to obtain a good  
222 estimation of the temporal evolution of the cyanobacterial abundance depends on the  
223 blooming species, *M. aeruginosa* or *A. flos-aquae*. Twice-monthly or monthly sampling  
224 provided good results for *M. aeruginosa*, whereas this was not often enough to monitor the  
225 chaotic population dynamics of *A. flos-aquae*. These findings are in contradiction with the  
226 recommendations of Codd et al. (1999), who proposed weekly or a twice-monthly sampling

227 for species that do not form scum (*A. flos-aquae* for example), and more frequent sampling  
228 for scum-forming species (such as *M. aeruginosa*), because they can display more rapid  
229 changes in concentration. On the other hand, in agreement with these authors, our findings  
230 also demonstrate that a reactive approach to cyanobacterial sampling is called for, and that  
231 appropriate monitoring programs must be devised for each ecosystem based on what is known  
232 about how these systems function. It is clear that sampling only once or twice a month can  
233 lead to a very considerable under-estimation of cyanobacterial concentrations, and thus of the  
234 health risks associated with the bloom. As a result, a weekly sampling frequency seems to be  
235 required for cyanobacteria in small freshwater ecosystems.

236         Our data on the variability of the spatial distribution of cyanobacteria in the lake  
237 indicate that at least three sampling points were needed to obtain a good estimation of the  
238 abundance, based on a comparison with estimations based on six sampling points. It appeared  
239 also that if only three sampling points are used, the choice of the location of these sampling  
240 points is very important for the quality of the estimation. The most reliable results were  
241 obtained using sampling points located on the opposite side of the lake shore to the main axis  
242 of the wind direction, and that adding more sampling points reduces the impact of the choice  
243 of the location of the sampling points. Such horizontal variability in the distribution of  
244 cyanobacteria has been previously documented for many ecosystems, and also for many  
245 cyanobacterial species. For example, in a recent study, Briand et al. (2009) showed that the  
246 spatial distribution of *M. aeruginosa* in a large freshwater reservoir on a given date could vary  
247 from  $7 \cdot 10^3$  cells/mL to  $2 \cdot 10^8$  cells/mL, depending on the location of the sampling points in the  
248 reservoir. Many factors and processes can influence the horizontal distribution of  
249 cyanobacteria in a freshwater ecosystem. Among them, wind and surface currents seem to  
250 have the greatest impact. For example, the distribution of *Microcystis* spp. in lake Taihu (see  
251 the review paper of Qin et al., 2010) and in Lake Ontario (Hotto et al., 2007) is clearly

252 influenced by both winds and currents. Similarly, Moreno-Ostos et al. (2009) have shown that  
253 in a Spanish reservoir currents have a marked effect on the distribution of cyanobacteria, and  
254 more globally on the phytoplankton community. In this study, we found that the horizontal  
255 distribution of *M. aeruginosa* in the lake was influenced more by wind direction than that of  
256 *A. flos-aquae*. This could be explained by the fact that *M. aeruginosa* colonies are located at  
257 the surface of the lake at the end of the night, and thus are more subjected to the influence of  
258 the wind than *A. flos-aquae* filaments, which are distributed over the entire water column. We  
259 found also that two sampling points in the lake (V5 and V6) were less influenced by wind  
260 direction than the others. This could be explained by the fact that these two sampling points  
261 are protected from the influence of winds blowing from the NW by an embankment located in  
262 the North part of the lake. Finally, we also demonstrated that in such a small lake, the impact  
263 of wind occurred at the scale of a few hours, in contrast to the previous findings of Welker et  
264 al. (2003) showing that the distribution of cyanobacteria was influenced by winds that had  
265 been blowing one or two days earlier.

266 In addition to this variability in their horizontal distribution; the vertical distribution of  
267 cyanobacteria was also variable. Indeed, during the 24 h for which we used the BBE Torch to  
268 monitor the concentrations of cyanobacteria, we found that they were lower in the subsurface  
269 layer early at night than during the day. The greatest variations in biomass were recorded  
270 during the daytime, both at the scale of one sampling point when the three measurements  
271 were compared, and at the scale of the five sampling points monitored during this study.  
272 These findings also suggest that several sampling points are necessary to obtain an accurate  
273 assessment of the cyanobacterial biomass and that integrated sampling of the first meter of the  
274 water column reduces the variability in the estimation of the biomass due to the position of  
275 cyanobacteria in the water column. This finding is consistent with data reported by Ahn et al.  
276 (2008) showing that an integrated method was the most appropriate sampling method for

277 *Oscillatoria* and *Microcystis* blooms. The causes of these variations in the position of  
278 cyanobacteria in the water column have been studied for different species. Several papers  
279 (Porat et al., 2001; Rabouille and Salençon, 2005; Rabouille et al., 2005; Visser et al., 2005;  
280 Walsby, 1994) have shown that migrations of cyanobacteria in the water column are probably  
281 due to the dynamics of the carbon-reserve metabolism, and are strongly influenced by light,  
282 temperature, and water mixing.

283 From all these findings, guidelines should be proposed for the monitoring of  
284 cyanobacteria in shallow lakes Codd et al. (1999) propose that the choice of sampling  
285 frequency and the choice of the number and location of the sampling sites should depend on  
286 the purpose of monitoring. For example, sampling near public bathing sites was  
287 recommended in freshwater ecosystems used for recreational activities. However, this  
288 strategy might generate data relevant only to the immediate vicinity of the bathing area, which  
289 do not reflect the global distribution of cyanobacteria in the lake. This is especially true when  
290 this distribution is very varied, and could make it difficult to prevent or manage blooms. On  
291 the basis of our findings, we proposed a different sampling strategy, which does not depend  
292 on the purpose of the monitoring. In order to minimize the cost of the cyanobacteria survey,  
293 twice-monthly sampling could be the norm for monitoring, but only if it is complemented by  
294 regular visual surveys. Changes in the appearance of the water (e.g. its color) between two  
295 successive dates would lead to an immediate increase in the sampling frequency. If it is not  
296 possible to carry out this visual survey, only a weekly sampling strategy can ensure that a  
297 sporadic cyanobacterial bloom is not missed. With regard to the number of sampling points,  
298 we found that at least three sampling points were necessary to obtain an accurate assessment  
299 of the cyanobacterial biomass (based on comparison with six sampling points). However,  
300 even when three sampling points were used, we found that the choice of the location of the  
301 sampling points was also very important (Fig. 8), even though the lake was fairly rectangular

302 in shape and its perimeter small (around 1.3 Km). These findings suggest that for large lakes  
303 and also for lakes with a more complex shape, a large number of sampling points would be  
304 necessary to obtain a good estimation of the cyanobacterial abundance. Clearly such sampling  
305 is time consuming and expensive. One way to reduce these costs would be to collect a large  
306 number of samples and then pool equal volumes of these samples in the same flask, before  
307 carrying out a single analysis. In this study, as in most of the monitoring programs performed  
308 in small lakes, all samples were taken from the shoreline of the lake. This kind of sampling is  
309 suitable for small lakes, but it has been shown that for large lakes (Rogalus and Watzin, 2008)  
310 shoreline sampling may miss early warning signs of bloom development, and also lead to the  
311 overestimation of the concentration of microcystins, when compared to data obtained from  
312 offshore samples. For bigger lakes, therefore, the sampling strategy must include offshore  
313 samples.

314 Different programs worldwide are testing alternatives to water sampling for the  
315 monitoring of cyanobacteria in freshwater ecosystems. Two main approaches have been  
316 investigated. The first one is based on the use of remote sensing, which has long been in use  
317 in marine ecosystems (see for example Bracher et al., 2009). In freshwater ecosystems, the  
318 paper of Hunter et al. (2008) has shown the potential of high resolution images for the  
319 assessment of the spatial distribution of *M. aeruginosa* in a shallow eutrophic lake. However,  
320 the cost of these images and the impact of meteorological conditions are limiting factors for  
321 envisaging the use of this tool in routine cyanobacteria monitoring programs. One alternative,  
322 lower-cost solution could be based, in the future, on the use of drones to take aerial  
323 photographs of freshwater ecosystems, but these tools are still in development. Moreover,  
324 they will be only useful for cyanobacterial species that live in the surface water of lakes.

325 The second way of monitoring of cyanobacteria without sampling the water being  
326 investigated is the use of buoys equipped with a variety of sensors, including, for example, a

327 submersible spectrofluorometer to quantify the biomass of the cyanobacteria. This kind of  
328 tool permits the real-time monitoring of phytoplankton, including cyanobacteria, as shown for  
329 example in the paper of Le Vu et al. (in press). The two obstacles to their use in routine  
330 cyanobacteria monitoring programs are the high price of these systems, and the fact that they  
331 only provide estimations for one sampling point. Despite this, the possible use of such buoys,  
332 combined with the spatial monitoring of cyanobacteria by water sampling looks very  
333 promising for surveying cyanobacteria in freshwater ecosystems.

## 334 **5 Conclusion**

335 The sampling of cyanobacteria in freshwater ecosystems is a hot topic, in particular in the  
336 context of programs for surveying these toxic microorganisms in ecosystems used for the  
337 production of drinking water or for recreational activities. Paradoxically, fewer studies deal  
338 with the impact of sampling strategies on the estimation of cyanobacterial cell abundances in  
339 freshwater ecosystems. In this study, we demonstrate that the choice of sampling strategy can  
340 lead to very different estimations of the cell abundances of two blooming species in a shallow  
341 lake and also that, depending on the cyanobacterial species involved, different sampling  
342 strategies are required to obtain a good estimation of their population dynamics. All these  
343 findings suggested that monthly or twice-monthly sampling strategies at just one sampling  
344 point do not allow to provide an accurate estimation of cyanobacterial abundances, and thus  
345 of the health risks associated with the presence of toxic species in aquatic ecosystems.  
346 Moreover, although promising new technologies are being developed for monitoring  
347 freshwater cyanobacteria, their cost and some other drawbacks mean that at present they  
348 cannot replace water sampling, which will remain the basis of most of these monitoring  
349 programs for the foreseeable future.

350

351



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355

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444 Table 1: Relationship between wind direction and high cell abundance recorded for  
445 *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* at the different sampling points. We  
446

447 Fig. 1: Geographical location of the study site in France (left), and of the sampling points in  
448 the lake (right)  
449

450 Fig. 2: Changes over time of the concentrations of *Microcystis aeruginosa* (top) and  
451 *Aphanizomenon flos-aquae* (bottom). These concentrations were estimated by calculating the  
452 average cell count for the six samples at each date. The error bars indicate the standard  
453 deviation.

454  
455 Fig. 3: Simulation of the change over time of *Microcystis aeruginosa* cell concentrations  
456 found using a weekly (top), twice-monthly (middle) or monthly sampling frequency (bottom),  
457 with lags for the first sampling day of zero days (-), 2 days (--), and 4 days (...) comparing to  
458 our first sampling day. The gray curve corresponds to the reference data.  
459

460 Fig. 4: Simulation of the change over time of the biomass of *Aphanizomenon flos-aquae*  
461 found using a weekly (top), twice-monthly (middle), or monthly sampling frequency  
462 (bottom), and with lags for the first sampling day of zero days (-), 2 days (--), and 4 days (...) comparing to our first sampling day. The gray curve corresponds to the reference data.  
463  
464

465 Fig. 5: Spatial distribution of two cyanobacteria, *Microcystis aeruginosa* and *Aphanizomenon*  
466 *flos-aquae*, in the lake at four sampling dates (July, 9, 17 & 23; August, 8)  
467

468 Fig. 6: Change over time in the coefficients of variation of the mean cell abundances of  
469 *Microcystis aeruginosa* (black triangle) and *Aphanizomenon flos-aquae* (white square)  
470 estimated at all six sampling points.

471

472 Fig. 7: Spearman correlation values between *Microcystis aeruginosa* (top) and  
473 *Aphanizomenon flos aquae* (bottom) cell abundances estimated from the mean values for all  
474 six sampling point values, and those estimated from only one, two, three, four or five of these  
475 six sampling points.

476

477 Fig. 8: Location of the sampling points providing the best (left) and worst (right) estimations  
478 of cyanobacterial cell abundances, compared to estimations based on six sampling points. We  
479 give the combinations for two (top) and three (bottom) sampling points. The polar plot shows  
480 the direction of the maximum daily wind speed during the study. The different line types  
481 permit to distinguish the two best or the two worst combinations of sampling points, using  
482 two or three sampling points.

483

484 Fig. 9: Cyanobacterial biomass in the subsurface water of the lake over a 24-hour period at  
485 five sampling points (◆ point A, ■ point B, ▲ point C, × point D, and ◇ point E). The error  
486 bars indicate the standard deviation.

487

488

489 Supplemental Figure 1. Distribution of the wind directions at the St-Etienne-Bouthéon  
490 meteorological station during this study (June, 13 to October, 10, 2008). The curve and the  
491 bars indicate respectively the mean speed and the occurrence per hour of the wind in each  
492 direction.

493

494 Supplemental Fig. 2. Evolution of the spatio-temporal distribution of *Microcystis aeruginosa*  
495 (left) and *Aphanizomenon flos aquae* (right) in the lake during our study (the scale is the same  
496 than in Fig. 5).

497

498 Supplemental Fig. 3. Relationship between the cell concentration and the coefficient of  
499 variation for *Microcystis aeruginosa* (top) and *Aphanizomenon flos-aquae* (bottom)

500